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Enterococci of animal origin and their significance for public health

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Abstract

Enterococci are commensal bacteria in the intestines of humans and animals, but also cause infections in humans. Most often, *Enterococcus faecium* isolates from clinical outbreaks belong to different types than *E. faecium* from animals, food, and humans in the community. The same variants of the *vanA* gene cluster (Tn*1546*) encoding vancomycin resistance can be detected in enterococci of both human and animal origin. This could indicate horizontal transfer of Tn*1546* between enterococci of different origin. *E. faecium* isolates of animal origin might not constitute a human hazard in themselves, but they could act as donors of antimicrobial resistance genes for other pathogenic enterococci. *Enterococcus faecalis* of animal origin seems to be a human hazard, as the same types can be detected in *E. faecalis* from animals, meat, faecal samples from humans in the community, and patients with bloodstream infections.

Keywords: *Enterococcus faecalis*, *Enterococcus faecium*, gene transfer, growth promoters, molecular typing
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Introduction

Enterococci are commensal bacteria in the intestines of humans and domestic animals, but they can also be detected in the environment, from soil, water, plants, wild animals, birds, and insects. In humans, *Enterococcus faecalis* and *Enterococcus faecium* can cause urinary tract infections, wound infections, bacteraemia, and infective endocarditis. Resistant enterococci are selected both in humans and in animals, owing to the use of antimicrobial agents in both settings.

In this review, enterococci of animal origin and their significance for public health are described. This includes the findings of vancomycin-resistant enterococci and streptogramin-resistant *E. faecium* outside of hospitals, a description of the persistence of vancomycin-resistant enterococci after the banning of avoparcin, comparison of enterococci of human and animal origin, and different models with which to study gene transfer between enterococci of different origin.

Clinical Background

Over the past two decades, *E. faecalis* and *E. faecium* have become increasingly important pathogens worldwide, especially because of life-threatening hospital-acquired infections, including bacteraemia and infective endocarditis [1]. Enterococcal bacteraemia is associated with high 30-day mortality rates.

Enterococci are intrinsically resistant to a number of first-line antimicrobial agents; they show low-level resistance to β-lactams, resistance to cephalosporins, and low-level resistance to aminoglycosides. Therefore, treatment of enterococcal infections may be difficult. Furthermore, enterococci can acquire resistance to other antimicrobial agents, including quinolones, macrolides, tetracyclines, streptogramins, and glycopeptides [1,2]. Most often, enterococcal infection has been treated with synergistic and bactericidal therapy with a combination of an aminoglycoside (gentamicin) and a β-lactam (or other cell wall agents, such as vancomycin). This will work as long as the organism does not exhibit high-level

resistance to the aminoglycoside, or resistance to β-lactams or to vancomycin, making this combination the standard of care for severe enterococcal infections [3]. Newer antibiotics such as linezolid, daptomycin and tigecycline have good *in vitro* activity against enterococcal isolates, although their clinical use may be limited in certain clinical scenarios as a result of reduced rates of success, possible underdosing for enterococci, and low serum levels, respectively, and also by the emergence of resistance [3]. *E. faecium* is among the so-called 'ESKAPE' pathogens (*E. faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumanii*, *Pseudomonas aeruginosa*, and *Enterobacter* species), which cause the majority of the infections in US hospitals and effectively 'escape' the effect of antibacterial drugs [4].

First Reports on Vancomycin-resistant Enterococci and Quinupristin–dalfopristin-resistant *E. faecium* Outside Hospitals

The first description of a non-human reservoir of vancomycin-resistant *E. faecium* was published in 1993 [5]. Bates *et al.* detected vancomycin-resistant *E. faecium* in farm animals in the UK, even though vancomycin never had been used for the treatment of animals. However, another glycopeptide, avoparcin, had, since the mid-1970s, been approved as an additive in feed for farm animals in many countries (although not in the USA and Canada). It was hypothesized that avoparcin was selecting for vancomycin-resistant enterococci in animals.

This hypothesis was tested with aimed studies in poultry flocks and pig herds fed with feed with or without avoparcin. These studies confirmed that avoparcin in the feed had a significant role in selecting for vancomycin-resistant *E. faecium* in the animals [6,7].

After the first paper from Bates *et al.*, several papers from many parts of the world described vancomycin-resistant enterococci from many different non-human sources, e.g. cats, dogs, horses, wild birds, foxes, wood frogs, ostriches, pigs, pork, broilers, poultry meat, environmental samples, and sewage, as well as from stool samples from farmers and non-hospitalized humans in the community. Most of the studies reported isolation of vancomycin-resistant *E. faecium* from the community, whereas only a limited number of studies detected vancomycin-resistant *E. faecalis* from non-hospital sources. In the mid-1990s, vancomycin was one of only a few antimicrobial agents remaining for the treatment of ampicillin-resistant and gentamicin-resistant *E. faecium* isolates causing life-threatening infections (e.g. bacteraemia and infective endocarditis), which were already common. It was therefore of major concern that large amounts of avoparcin were used as feed additives for animal production, thereby selecting for vancomycin-resistant enterococci. In 1994, 24 kg of active vancomycin was used for human therapy in Denmark, whereas 24 tons of avoparcin was used for pig and broiler production [8]. From 1992 to 1996, Australia imported an average of 582 kg of vancomycin per year for human medical purposes, and 62.6 tons of avoparcin per year for animal production [9].

In 1997, Woodford *et al.* [10] reported the finding of quinupristin–dalfopristin-resistant *E. faecium* in non-hospitalized humans in the UK. Quinupristin–dalfopristin and other streptogramins used for human therapy were not licensed in the UK at that time for use in humans. The finding of quinupristin–dalfopristin-resistant *E. faecium* isolates outside hospitals was assumed to be associated with the use of virginiamycin for animals [11–13]. The *vat*(D) and *vat*(E) genes, encoding streptogramin A resistance in *E. faecium*, have been detected in *E. faecium* isolates from poultry, pigs, pork, sewage and animal manure in Europe, Asia and the USA. Recently, two new streptogramin A resistance genes, *vga*(D) and *vat*(H), have been detected in *E. faecium* isolates from healthy humans, pigs, poultry and chicken meat in Korea [14].

On the basis of the precautionary principle, regarding concerns about human health, the use of avoparcin was banned in Denmark and Norway in 1995, in Germany in 1996, and in the rest of the EU in 1997. Avoparcin was banned in Korea in 1997, and in Taiwan and New Zealand in 2000 [15–17]. Like avoparcin, virginiamycin was banned in Denmark in 1998 and in all of the EU in 1999 on the precautionary principle. In Australia, its use was restricted to therapeutic purposes in 2008.

The use of antimicrobial agents for growth promotion was banned in all EU countries in 2006, but antimicrobial agents are still used for the treatment of animals. Tetracycline, in particular, is used to a great extent in animal production. Even though tetracycline is not used for the treatment of enterococcal infections in humans, it is important for the selection of resistant enterococci, as tetracycline-resistant enterococci are often resistant to other antimicrobial agents (e.g. vancomycin and gentamicin).

Like tetracycline, tylosin (a macrolide) is used for the treatment of diseases in pigs. The *erm*(B) gene encodes resistance to both tylosin and erythromycin. The use of tylosin might be related to the persistence of vancomycin-resistant enterococci in pigs; this is described in detail below [18].

Persistence of Vancomycin-resistant *E. faecium* After the Banning of Avoparcin

Several studies from countries around the world (e.g. Denmark, Norway, Portugal, Taiwan, Korea, and New Zealand) have shown that vancomycin-resistant *E. faecium* persisted in animals for an extended time after the banning of avoparcin. Vancomycin-resistant *E. faecium* isolates with indistinguishable or highly similar pulsed-field gel electrophoresis (PFGE) profiles were obtained from consecutive broiler flocks reared in the same house, and from environmental samples obtained in the houses in between the flocks. In contrast, isolates from different broiler houses and from flocks reared in different houses appeared to be genetically unrelated. These findings indicated that vancomycin-resistant *E. faecium* isolates were transmitted between consecutive broiler flocks by clones of resistant bacteria surviving in the broiler houses despite cleaning and disinfection between production cycles [19]. The same clones of vancomycin-resistant *E. faecium* were detected in Danish pigs and in healthy humans [18,20]. Thirteen years after the banning of avoparcin, the same clones could be detected in pigs (A. M. Hammerum, unpublished data).

One of the explanations for the persistence of vancomycin-resistant *E. faecium* is co-selection with other antimicrobial agents or metals. In the Danish pig industry, vancomycin-resistant *E. faecium* persisted in faeces from pigs at a frequency of approximately 20% until the use of tylosin was reduced; thereafter, the occurrence of vancomycin-resistant *E. faecium* decreased to only a few per cent [18]. The persistence could be explained by co-selection with tylosin, as *erm*(B) and *vanA* were located on the same plasmid [18]. Likewise, copper sulphate (used as a growth-promoting feed supplement for pigs) was selecting for vancomycin-resistant *E. faecium*, as the copper resistance gene *tcrB* was found on a plasmid containing both *vanA* and *erm*(B) [21]. Furthermore, many of the vancomycin-resistant *E. faecium* isolates are also resistant to tetracycline, which is also used for animal production. Tetracycline might therefore co-select for vancomycin-resistant *E. faecium* as well.

Johnson *et al.* have recently used Danish surveillance data in a mathematical model for the persistence of vancomycin-resistant *E. faecium* in the Danish broiler industry. These analyses suggested that acquired vancomycin resistance would persist for more than 25 years—until 2036 [22].

Comparison of Enterococcal Isolates of Animal Origin with Enterococcal Isolates of Human Origin

Since the detection of vancomycin-resistant enterococci and streptogramin-resistant *E. faecium* outside hospitals, different molecular typing methods have been used to compare enterococci obtained from different sources. In some studies, genetic profiles were compared to investigate clonal transfer between enterococci. This is described below, as are other studies that compared the *vanA* transposon from different reservoirs.

In the first typing studies, PFGE was used for comparison of *E. faecium* isolates of animal origin with *E. faecium* isolates of human origin. Similar and highly similar PFGE profiles were found for vancomycin-resistant animal isolates and human stool isolates, respectively [20,23].

Later, amplified fragment length polymorphism analysis and multilocus sequence typing (MLST) were used for comparison of *E. faecium* isolates of different origin. The first amplified fragment length polymorphism study led to the conclusion that vancomycin-resistant *E. faecium* strains were predominantly host-specific, and strains isolated from hospitalized patients were genetically different from the prevailing vancomycin-resistant *E. faecium* strains present in the faecal flora of non-hospitalized humans [24]. The first MLST studies showed that outbreak isolates from hospitalized humans clustered in a subgroup, clonal complex (CC)17, whereas *E. faecium* isolates of animal origin belonged to other sequence types (STs) and CCs [25,26]. The CC17 *E. faecium* isolates have been found in at least five continents; they most often show ampicillin resistance and high-level ciprofloxacin resistance, and many show vancomycin resistance and carry specific virulence genes [24,27]. *E. faecium* isolates were therefore thought to be host-specific. Later studies have shown that dogs can be a reservoir of *E. faecium* isolates belonging to STs related to isolates from clinical infections or hospital outbreaks with ST17 as the primary founder [28,29]. However, it is important to note that the dogs in the study by Ghosh *et al.* [29] had all been at a veterinary intensive-care unit, and so might have differed from the normal healthy dog population. A few other studies have detected *E. faecium* isolates of animal origin belonging to CC17: ST132 (part of CC17) isolates were obtained from a pig and from a human urinary tract infection [30]; a *vanB2* ST17 *E. faecium* isolate was obtained from chicken meat and an *E. faecium* isolate was obtained from veal in a Spanish study [31]; and a *vanA* ST78 *E. faecium* isolate was obtained from rabbit meat [31].

E. faecium isolates from pigs most often belong to a single cluster (CC5) by MLST. *vanA E. faecium* isolates belonging to CC5 have been found in pigs from five European countries and the USA [30]. Furthermore, CC5 *E. faecium* isolates have also been found in patients with urinary tract infections and in faecal samples from non-hospitalized humans [30].

More recently, *E. faecalis* isolates from different sources have been compared; as for *E. faecium*, *E. faecalis* outbreaks in hospitals have been related to specific MLST clonal complexes (CC2, CC9, and CC87). CC2 (ST6) has also been detected outside hospitals in pigs and in healthy infants [32,33]. Other STs seem to be more widespread; for example, ST16 *E. faecalis* isolates have been detected in pigs, poultry, healthy humans, and patients [32–35]. Furthermore, *E. faecalis* isolates with high-level resistance to gentamicin belonging to ST16, and with similar PFGE types, have been obtained from pigs, pork, non-hospitalized humans, and patients with endocarditis [36]. ST116 was found in *vanA E. faecalis* isolates from turkey meat, non-hospitalized humans, and a patient [37]. *E. faecalis* isolates of ST40 and ST97 have been detected in both pigs and endocarditis patients [38].

The *vanA* gene is located on a 10 851-bp transposon, named Tn*1546*. It encodes nine polypeptides that can be assigned to various functional groups: transposition (ORF1 and ORF2), regulation of resistance gene expression (VanR and VanS), synthesis of the D-Ala-D-Lac depsipeptide (VanH and VanA), and hydrolysis of peptidoglycan precursors (VanX and VanY). The function of VanZ remains unknown. Several groups have characterized and compared Tn*1546* transposons from different sources to investigate the possible horizontal transfer of *vanA* between enterococci of animal origin and enterococci of human origin.

Even though Tn*1546* transposons in most of the studied enterococci were heterogeneous, several groups detected the same variant of Tn*1546* in enterococci of human and non-human origin [39–42]. Furthermore, Jensen found a point mutation in *vanX* (G to T) at position 8234 in Tn*1546*. The variant with a T was associated with a pig origin of the vancomycin-resistant *E. faecium* isolates, whereas a G in position 8234 was detected in isolates of poultry origin. Both types were found among human *E. faecium* isolates [39,43].

In conclusion, *E. faecium* isolates from clinical outbreaks most often belong to different types than *E. faecium* from animals, food, and humans in the community. Even though *E. faecalis* isolates from hospital outbreaks also belong to specific types, the same MLST types can be detected in *E. faecalis* isolates from animals, meat, faecal samples from humans in the community, and patients with bloodstream infections. The same variants of Tn*1546* can be detected in enterococci of both human and animal origin. This indicates the possibility of horizontal transfer of Tn*1546* between enterococci occupying various ecological niches.

Can *vanA* be Transferred Between Enterococci of Animal and Human Origin in the Intestine?

The intestines of animals, including humans, are ideal places for gene transfer, and several models have been used to study gene transfer between enterococci in the intestine. These models include gnotobiotic mice/rats and gnotobiotic mice with a human microbiota; gene transfer has also been studied in the intestines of healthy humans.

Two different studies showed transfer at a high frequency of *vanA* from an *E. faecium* isolate of animal origin to an *E. faecium* isolate of human origin in the intestine of gnotobiotic mice [44,45]. Mater *et al.* and Bourgeois-Nicolaos *et al.* observed transfer of *vanA* from an *E. faecium* donor to an *E. faecium* recipient in the intestines of gnotobiotic mice with human faecal flora [46]. Transfer of *vanA* from an *E. faecium* isolate of animal origin to an *E. faecium* isolate of human origin has also been investigated in the intestines of healthy human volunteers. *In vivo* conjugation occurred in three of six volunteers [47]. In one volunteer, co-transfer of several resistance genes occurred. The *vanA* gene was transferred together with *vat*(E) and *erm*(B) (encoding streptogramin and macrolide resistance, respectively). The recipient strain in the above-mentioned study by Lester and Hammerum [48] did not belong to CC17. In a recent study, *vanA* of animal origin was transferred to a CC17 recipient (obtained from a patient with sepsis) in the intestines of cephalosporin-treated mice. This study shows that, even though *vanA* CC17 *E. faecium* isolates are associated with hospital outbreaks, the *vanA* genes in these isolates could have an animal origin.

Lim *et al.* [49] found very similar plasmids from humans and chickens, indicating gene transfer between different hosts. Furthermore, Sletvold *et al.* compared a *vanA E. faecium* plasmid from a farmer with a *vanA E. faecium* plasmid from his poultry. The two plasmids shared 43 coding sequences, and the only nucleotide difference was an 88-bp indel [50].

Most Tn*1546* transposons are plasmid-borne. The studies described above illustrate that horizontal transfer of *vanA* (Tn*1546*) between enterococci of different origin can occur in the intestine.

Are Enterococci of Animal Origin a Human Hazard?

Transient colonization with enterococci of animal origin has been shown in the intestines of healthy humans not receiving antimicrobial agents for between 4 to 30 days [51,52]. These resistant enterococci may act as donors of resistance genes (e.g. *vanA* and *vat*(E)). Lester and Hammerum showed this transfer to CC17 *E. faecium* in the intestines of cephalosporin-treated mice, whereas, in the study by Lester *et al.*, gene transfer in the intestines of healthy humans was investigated without antimicrobial treatment, because of ethical considerations. Gene transfer of *vanA* may occur in the intestines of human patients, from an *E. faecium* strain of animal origin (obtained prior to the hospital stay or from eating meat containing resistant *E. faecium* strains during the stay) to a hospital CC17 *E. faecium* strain obtained during the hospital stay. One of the major risk factors in relation to colonization or/and infection with enterococci is antimicrobial treatment. Ubeda *et al.* [53] have shown, by 16S DNA pyrosequencing, that antimicrobial treatment can disrupt the microbiota, enabling vancomycin-resistant enterococci to undergo dramatic expansion and thereafter dominate the microbial population of the ileum and caecum. In the clinical setting, Ubeda *et al.* [53] found that intestinal domination by vancomycin-resistant enterococci preceded bloodstream infections in patients undergoing allogeneic haematopoietic stem cell transplantation. In conclusion, *E. faecium* strains of animal origin most often do not constitute a human hazard in themselves, but they can act as donors of antimicrobial resistance genes for other pathogenic enterococci.

The situation seems to be different for *E. faecalis* of animal origin. Larsen *et al.* [36,38] found *E. faecalis* isolates from human patients and pigs with highly similar profiles in relation to resistant pattern, virulence gene profile, and MLST/PFGE types. This indicates that *E. faecalis* from pigs might constitute a human hazard.

It is hard to quantify this risk for both *E. faecium* and *E. faecalis* of animal origin in relation to human health, and further studies are needed.

Further Perspectives

Enterococci can survive and live in harsh environments, and are therefore hard to eradicate in both animal production and clinical settings. Proper cleaning of animal production facilities, such as according to the 'all-in/all-out principle' (working in the poultry houses), can minimize the persistence of enterococci in poultry houses. In slaughterhouses, good hygiene is also essential to minimize faecal contamination of the meat with enterococci and other zoonotic bacteria. Similarly, good hand hygiene and proper cleaning is crucial in the clinical setting to avoid nosocomial infections with enterococci.

Even though the use of growth promoters is banned in all EU countries, avoparcin and virginiamycin are still used in other parts of the world. Furthermore, other antimicrobial agents used in animal production for therapy can select for vancomycin-resistant enterococci, owing to co-resistance. Prudent use of antimicrobial agents in animal production is therefore essential to lower the risk of selection of resistant enterococci or other bacteria with a zoonotic potential (methicillin-resistant *S. aureus*, resistant *Escherichia coli*, and resistant *Salmonella* species). In the human clinical setting, prudent use of antimicrobial agents is also needed to decrease the number of nosocomial enterococcal infections, as antimicrobial agents constitute a risk factor for infections with enterococci.

Antimicrobial resistance can easily be transferred between borders, because people travel, and meat and livestock are exported. Antibiotic resistance is therefore not only a national problem, but also a global problem. A global policy on the prudent use of antimicrobial agents for both human and animal infections is therefore required to avoid the spread of resistance; the use of antimicrobial agents for growth promotion should be stopped in all countries around the world.

Transparency Declaration

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